

Oviposition Site Selection of the Plateau Frog (*Nanorana pleskei*) in the Zoige Wetland, China

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Abstract For oviparous species, oviposition site selection influences adult reproductive success as well as the fitness of the resulting offspring. Females usually choose potential egg-laying sites depending on abundance and quality to maximize their reproductive success. We focused on the oviposition site selection of this plateau frog in Zoige wetland and investigated how the egg-laying pattern of the females influences their offspring's survival. We found that shallow waters, decentralized spawning patterns, and egg attachment to appropriate distance to the water surface were the main spawning strategies of *Nanorana pleskei* endemic to Qinghai-Tibet Plateau. We argued that drought caused by increasing temperature and variable precipitation has probably influenced *N. pleskei* reproductive success, which may be a crucial reason for its population decline. Our findings have important significance for habitat preservation, increasing embryo survival and establishing practical conservation policies.

Keywords oviposition site, metamorphic success, *Nanorana pleskei*, Zoige wetland

1. Introduction

Oviposition site selection means females choose particular places to lay their eggs instead of a random location (Wilson, 1998), this behavior functions to ensure that eggs avoid predation and environmental extremes (Becker and Erdelen, 1982; Joern and Jackson, 1983; Petranka, 1990; Burger, 1993). Because most amphibians do not provide egg attendance, egg thermoregulation and nursing, or other kinds of post-ovipositional care (Huang, 1990; Pough *et al.*, 2003), oviposition site selection hence is directly responsible to embryonic and larval survival, growth and development (Kolbe and Janzen, 2001; Wilson, 1998).

Obtaining oxygen is crucial for amphibian embryo

survival and development (Pinder and Friet, 1994; Seymour and Roberts, 1995; Seymour *et al.*, 2000). Amphibians generally lay eggs in aquatic environments and tend to attach their capsules to hydrophytes, thus placing them close to the water's surface to aid in embryonic oxygen exchange (Seymour, 1999). But for embryos and tadpoles of aquatic breeding amphibians, drought is a high risk when they were laid in shallow pools or short distance range to the water surface. As a result, amphibians must trade-off between two environmental pressures (hypoxia and drought), the distance to the water surface and depth of the water body. Zoige wetland is located in the eastern boundary of Qinghai-Tibet Plateau with average elevation at 3500 m a.s.l., where low oxygen density creates a hypoxic environment for all native wildlife.

The plateau frog (*Nanorana pleskei*) is endemic to the Qinghai-Tibet Plateau at altitudes of 3300 to 4500 m a.s.l. The habitats for this species are marshes, pools, and ponds in open (Fei *et al.*, 2009). This species was widely distributed in the Zoige wetland 20 years ago, but recent

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surveys indicate that the plateau frog's population has declined significantly in this region (Fellers *et al.*, 2003). However, studies focused on *N. pleskei*'s oviposition site choice were merely qualitative (Fei *et al.*, 2009).

The aims of our study were to: 1) record the oviposition site selection of the plateau frog in Zoige wetland; 2) test if the egg-laying pattern of the females influences their offsprings' survival; 3) investigate the relationship between the oviposition site selection and the plateau frog's population dramatically declining. We hope our outcomes may aid future endeavors related to habitat preservation, increasing larvae survival and establishing practical conservation policies.

2. Materials and Methods

2.1 Study site The study area (33.57° N, 102.48° E, 3450 m a.s.l.) is a wetland located in Xiaman village of Zoige National Nature Reserve of Sichuan Province, China (Figure 1). Zoige wetland is one of the largest plateau peat bogs in the world and located in the northeast of the Qinghai-Tibet Plateau. It is also an important area for carbon sinks and carbon sources around the world,

with high biodiversity due to its abundant animal and plant resources (Hao *et al.*, 2008; Wang and Tian, 2015). Meantime, Zoige wetland is one of the most sensitive areas to global climate change (Wang *et al.*, 2008).

2.2 Survey methods We surveyed the wetland by line transect sampling from 20 May to 10 July, 2015. We have set our line transects in alpine grassland vegetated by *Festuca nivina*, *Kobre siasetchuanensis* and *Elymus nutans*, mainly Cyperaceae spp. and Gramineae spp., herbaceous plants. Our line transects were set in a typical alpine meadow. We have set four line transects, each line-transect was 4 meters wide, 500 meters long and set in wetland. To avoid interference on the animals, we surveyed our line transects every 5 days. We recorded the number of amplexant pairs and marked 28 fresh clutches of *N. pleskei* by establishing a wood stick in the puddle near these clutches and coded them according to discovery sequence by writing the number on the wood stick with a mark pen. Four parameters of the clutch were measured: clutch size, the number of egg masses, the depth of oviposition puddle, and the distance from eggs to water surface. Clutch size and the number of egg masses were counted by eye (Figure 2). The depth of oviposition puddle is the distance from the water bottom to surface. The distance from the clutch to water surface is the mean value of the distances from every contained egg masses to water surface, we measured these distances from water surface to the top egg in each egg mass by digital caliper. In each survey, only fresh clutches with transparency and without expanded egg capsule (earlier than 8 cell stage) were measured. Furthermore, we followed these clutches continuously until hatched larvae finished metamorphosis, and recorded whether the embryos were healthy developed. The aggregation extent of eggs was defined as clutch size divided by the number of egg masses in the same clutch.

We used one-sample Kolmogorov-Smirnov Test to examine whether the data of the four parameters is in normal distribution and the Spearman correlation coefficient to represent the relationship between clutch size and the aggregation extent of eggs, the distance from eggs to water surface and the aggregation extent of eggs, and the depth of oviposition puddle and the aggregation extent of eggs, respectively. The metamorphic ratio was defined as clutches that completed their metamorphosis divided by all marked clutches.

All statistical analyses were conducted using IBM SPSS 21.0 Software (SPSS, 2012, IBM corporation, New York). All tests were two-tailed with a significance level (*P*) of 0.05.

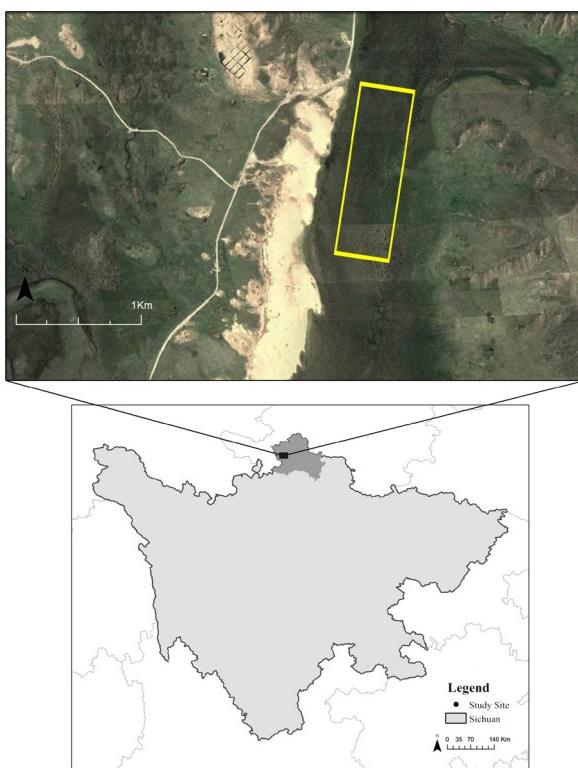


Figure 1 The map of survey sites in Zoige National Nature Reserve of Sichuan Province, China.



Figure 2 Photo of egg clutch of *Nanorana pleskei* and the black arrow shows egg masses (Photo by Gang WANG).

3. Results

In total, thirty-eight amplexant pairs of *N. pleskei* were found in the field. The breeding season of *N. pleskei* lasted from late May to early July and peaked in early June in Zoige wetland (Figure 3).

Clutch size of *N. pleskei* varied greatly among different female individuals, and the average clutch size was 96 ± 33 , $n = 28$, ranging from 35 to 159. The aggregation extent of eggs and the distance from eggs to water surface showed a significantly negative correlation ($n = 28$, $r = -0.52$, $P = 0.004$, Figure 4), which indicated that the eggs were further to the water surface (Table 1). Moreover, there was limited relationship between the aggregation

extent of eggs and the depth of oviposition puddle ($n = 28$, $r = 0.21$, $P = 0.29$). The aggregation extent of eggs and clutch size were also limited relevant ($n = 28$, $r = 0.35$, $P = 0.065$).

Inconsistent with the egg mass description in Fei *et al.* (2009), all clutches were found under the water surface, but no floating eggs were observed. The distance between eggs and water surface ranged from 16.10 to 74.81 mm, the females mainly selected medium distance range (30 to 50 mm) to lay their eggs, 61% of the observed clutches were located in this range (Figure 5). Females preferred to select shallow water to lay their eggs, the depths of spawning pools were from 56.61 mm to 105.35 mm.

The metamorphic ratio was extremely low. More than

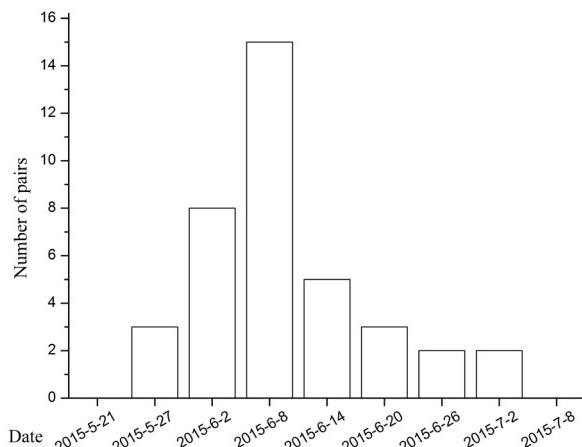


Figure 3 The number of amplexant pairs observed in Zoige, China.

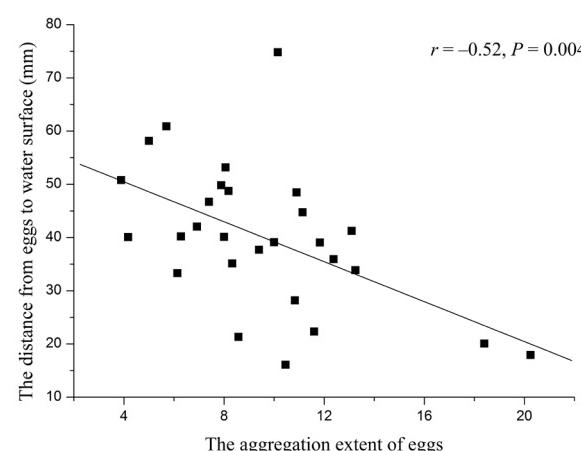


Figure 4 The relationship between the aggregation extent of eggs and the distance from eggs to water surface.

Table 1 Spawning pattern of *Nanorana pleskei* collected in Zoige, China.

Serial number	Time	Clutch size	Number of egg masses	Depth of oviposition puddle (mm)	Distance from eggs to water surface (mm)
1	2015-6-8	116	10	52.63	22.32
2	2015-6-8	65	6	51.61	28.18
3	2015-6-8	136	13	55.40	16.10
4	2015-6-8	78	7	59.29	44.74
5	2015-6-8	142	12	88.06	39.05
6	2015-6-8	81	4	94.40	17.93
7	2015-6-8	99	8	93.54	35.92
8	2015-6-8	110	11	80.58	39.11
9	2015-6-8	53	6	88.04	35.12
10	2015-6-8	74	10	84.29	46.70
11	2015-6-8	144	11	81.42	41.23
12	2015-6-8	66	14	63.67	40.08
13	2015-6-8	103	12	75.60	21.33
14	2015-6-8	94	10	90.67	37.70
15	2015-6-14	83	12	80.54	42.05
16	2015-6-14	129	16	103.83	53.17
17	2015-6-14	71	9	89.35	49.83
18	2015-6-14	109	10	88.69	48.46
19	2015-6-14	43	7	58.46	33.30
20	2015-6-14	57	10	87.08	60.90
21	2015-6-14	35	9	55.83	50.79
22	2015-6-14	110	22	91.06	58.16
23	2015-6-14	132	13	85.67	74.81
24	2015-6-14	159	12	105.35	33.86
25	2015-6-14	64	8	57.88	40.11
26	2015-6-14	139	17	75.40	48.75
27	2015-6-14	113	18	63.68	40.19
28	2015-6-14	92	5	70.17	20.07

half of the 28 marked clutches have hatched tadpoles in 10–12 days, but only four clutches' tadpoles finished their metamorphosis at the end of August. The metamorphic ratio was only 14.3%. The other clutches were all perished because of drought caused by no rainfall for more than half a month.

4. Discussions

Amphibians tend to lay large and constrictive egg

masses in oxygen-rich water, but small and separate ones when oxygen is insufficient (Moore, 1940; Zweifel, 1968). If the egg masses are near the water surface, the aggregation extent will be high, because upper water has relatively rich oxygen content, while the egg masses in lower water have low aggregation extent with relatively lower oxygen content. Our data showed a significantly negative relationship between the aggregation extent of eggs and the distance from eggs to water surface,

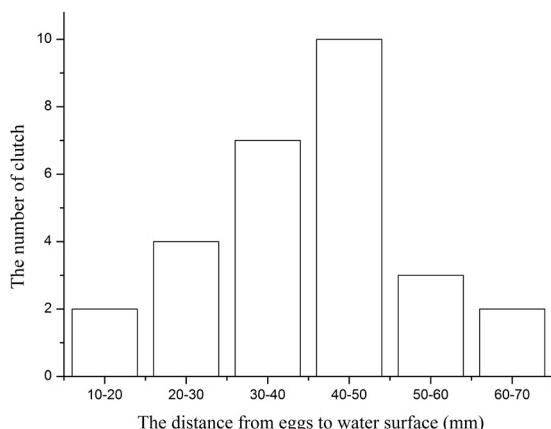


Figure 5 The distribution of distance from eggs to water surface in different clutches.

which indicated that oxygen is a crucial selective pressure for *N. pleskei* in choosing oviposition sites. To ensure the embryo gets sufficient oxygen support during development, females tend to place the clutch near the water surface (Seymour, 1999). However, the closer clutches are to the water surface, the higher their risk of desiccation if the water level lowers. Oviposition site selection is an important aspect of reproduction for oviparous species (Snider and Janzen, 2010) as it can affect reproductive success, specifically, embryo survival, hatching success, and larval development (Figiel and Semlitsch, 1995; Rudolf and Rödel, 2004; Snider and Janzen, 2010). It is critical for species that breed in dynamic environments and, in particular, for amphibians that breed in ephemeral wetlands (Goldberg *et al.*, 2006). In our study, metamorphic success ratio of *N. pleskei* was 14.3% in the field. The metamorphic success ratio of this frog that comes from the same population was 50% in the laboratory (Wang unpublished). We deduced that increased temperature and unstable precipitation during our experimental cycle may cause many eggs and larvae died of losing necessary water support. While the other two sympatric anuran species in Zoige wetland, *Rana kukunoris* and *Bufo gargarizans* prefer laying their eggs in deeper and larger ponds, which makes the climate change had less influence on their eggs and tadpoles, so their population decline are not as notable as *N. pleskei* (Fellers *et al.*, 2003; Wang unpublished).

The dissolved oxygen content in the waters of Qinghai-Tibet Plateau showed a negative correlation with elevation. According to our results, *N. pleskei* in Zoige wetland usually chooses shallow pools (56.61 to 105.35 mm) as oviposition sites. Thus, eggs laid in shallow waters could more easily get oxygen at high

altitudes. Avoiding oviposition in deep waters was in accordance with the fact that egg development needed both oxygen and optimal temperature (Lin, 1958). The low oxygen content and slow rise of water temperature were not conducive to the egg development in deep water environment, which mostly accounted for *N. pleskei* avoiding laying eggs in deep water environment. Moreover, our field investigation found that *N. pleskei* hardly laid eggs in deep ponds, although there had a great deal of aquatic plants available for eggs attachment. The possible reason could be the risk of falling into the water bottom, which could lead to the embryo death from starving oxygen (Kluge, 1981; Seymour and Bradford, 1995).

In conclusion, shallow waters, decentralized spawning patterns and eggs attachment to appropriate distance to the water surface were the main spawning strategies of *N. pleskei* adaptive to Qinghai-Tibet Plateau. The spawning pattern was formed in the long term process of nature selection and adapted to the local climate. Climate change will greatly impact the spawning pattern of *N. pleskei*, offspring survival and thus the populations. How to preserve those shallow waterbody in the wetland will be the main problem to the plateau frog conservation under the global climate change. On what aspect and to what degree will the climate change effects the spawning pattern, offspring survival and continuation of population of *N. pleskei* needs further studied.

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